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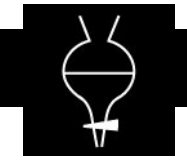
MULTIRESIDUE METHODS: EXTRACTION



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Introduction

'Killing two birds with one stone' is a common expression that captures the essence of multiresidue methods of analysis. Multiresidue methods are almost always more efficient than separate single analyte methods for multiple analytes. However, a possible drawback of multiresidue methods that cover a wide polarity range or diversity of analytes is a potential loss of selectivity for individual analytes. The use of high efficiency analytical separation techniques and/or very selective detectors can compensate for a lack of selectivity in preceding steps, but as a general rule, a greater degree of selectivity leads to higher quality results. Multiresidue methods often involve a balancing act between the analytical scope of the method and the quality of the results for all analytes. It is sometimes difficult 'to have your cake and eat it, too'.

Residues

In general, residues consist of synthetically derived chemicals that are not intended to occur in the sample, but may be present at trace concentrations as a by-product of a preliminary process related to the sample, or as a separate process altogether. Residues may be inorganic or organic, but inorganic compounds are generally analysed separately from organics. Multielemental analysis measures the natural occurrence of elements as well as any residues that may occur in the sample. Microorganisms and dirt may also be considered residues according to some definitions, but their analysis requires different techniques from organic compounds and they will not be considered further in this discussion. In the case of organic chemicals, many natural components are capable of being analysed in the same approach as the residue method, but these compounds are usually termed interferences, and great effort is often spent trying selectively to remove or avoid them (however, other chemists may be very interested in these matrix interferants).

The most common type of multiresidue application is the analysis of organic chemical contaminants in food and environmental samples. There are instances

when a residue is intended, such as a fungicide designed to extend product life, but for the large majority of situations, residues are not desired in the sample. Residues may consist of pesticides, drugs, industrial by-products and/or other pollutants. Within each of these categories are subcategories known as classes of analyte. For example, classes of insecticides include organophosphorus (OP), organochlorine (OC), carbamate, pyrethroid, and others; classes of pollutants include volatile organic compounds (VOC), polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB) alkylphenol ethoxylates (APE) and others; and veterinary drugs include antibiotics (β-lactams, aminoglycosides and tetracyclines), antibacterials (nitrofurans, fluoroquinolones and sulfonamides), and anthelminthics (benzimidazoles, avermectins and milbemycins). Thus, multiresidue methods may be single-class or multiclass depending on the number of analytes and classes covered by the analytical scope of the method.

A few existing multiresidue methods may be used to analyse more than one type of residue (e.g. pesticides and industrial pollutants), but most applications generally require the analysis of a single residue category. Analytical needs do exist that would entail the monitoring of all types of residues in some sample matrices (e.g. certain foods), but different methods are usually conducted for the separate analysis of the different residue types. It is difficult enough to develop and perform multiclass, multiresidue methods, and very few multi-type, multiclass, multiresidue methods have been attempted. However, some overall analytical schemes may include a wide array of analyte types in the extraction procedure and then divide the extract into separate aliquots for different clean-up and analytical steps.

The Analytical Process

The analytical process goes through a series of steps which leads to the analytical results. Much like a chain that is only as strong as its weakest link, an analytical method is only as good as its weakest step. These step consist of: (i) sample collection and handling; (ii) sample preparation (extraction, clean-up); (iii) analyte determination (analytical separation, detection); and (iv) reporting of results.

The primary consideration that must be addressed independently of the analytical steps, however, is the need for the data. The analytical method should be tailored to meet the minimum needs in terms of the scope of analytes to be detected, desired limits of detection (LOD) and acceptable precision and accuracy of the results. In many cases, the data needs will require the best possible results for as many analytes as possible. However, no analytical method can detect all possible analytes in the same procedure, and all laboratories are constrained by available personnel, space, instrumentation and other resources. Thus, the analytes must be prioritized according to importance and weighed against the cost and availability of analytical methods for their detection. The analytical chemist considers the most efficient overall approach to determine the analytes of interest that meets the acceptable data quality requirements and fits within the laboratory budget. This process may involve trade-offs between the quality of results for a particular analyte balanced against the quality of results for one or more other analytes.

The first step in the analytical process involves sample collection and handling. Unless representative samples are collected of the appropriate matrix and the samples are treated properly to avoid losses of analytes or potential contamination, then the results may not provide the information necessary to meet the needs for the analysis. In fact, a false sense of security in misleading results is often the outcome unless each step in the analytical process is carefully considered and controlled. For example, excellent recoveries and reproducibilities may be achieved for the analysis of several OP insecticides in liver tissue, but nearly all OPs partition into fat tissue in animals, and very few appear in the liver. If the purpose of the analysis is to determine animal exposure to OPs and assess risk to humans, then fat tissue should be sampled, not liver. Otherwise, the analysis may accomplish nothing of real significance.

Extraction

Once the appropriate sample has been collected and handled properly, the next sequential step in the analytical process is the sample preparation procedure which is the subject of this article – *extraction*. Extraction is the separation of the analyte from the matrix. A few techniques, such as direct analysis of chemicals in liquids, may avoid the extraction step, but in most cases, the analytes must also be concentrated prior to analysis, which often necessitates an extraction process. The post-extraction steps in the analytical process may include clean-up of extracts and an analytical separation, but some sample preparation techniques incorporate clean-up and analyte separation into the extraction procedure. In other cases, the final analyte detection step may not require extra clean-up or analytical separation steps, but usually the price to pay for an approach that avoids clean-up steps is reduced ruggedness and higher instrument maintenance.

The type of extraction step that is used for a particular matrix depends on the nature of the matrix and analytes. Fundamentally, typical samples consist of solids, liquids and gases, or combinations thereof. Solids are usually not reasonably extracted with solids, and gases may not be extracted with gases (unless a solid membrane is placed between them), but all other combinations of liquid–solid, solid–gas, liquid–liquid and liquid–gas extractions are common in extraction techniques; supercritical fluids have also become a useful medium for extraction processes. With modern extraction techniques and the number of different solvents available, the control of pressure and temperature in the separation process has provided an essentially limitless number of possible separation conditions for the chemist to employ.

Extractions Involving Gases

For gaseous samples, the extraction process nearly always involves passing large volumes of sample through a solid phase or liquid trap. In solid-phase systems, the analytes are adsorbed on to the particle surfaces, and in liquids the analytes are partitioned into the liquid. In the case of low temperature trapping, the analytes may simply condense on to the cold surfaces. For the most volatile analytes, a combination of condensation and adsorption may be conducted by maintaining low temperature of an adsorptive surface. The analytes are concentrated in the trapping medium and may undergo clean-up or be directly analysed in a chromatographic system. The most common approach is probably to use active materials, such as Tenax, polyurethane foam, octadecylsilyl-derivatized silica, polymer resins, or a variety of other materials to adsorb the analytes.

The use of liquid trapping by bubbling the gas through a liquid is another easy approach to extracting air-borne substances, but evaporative losses of the liquid, greater temperature limitations and less convenience generally make large volume liquid trapping a less common approach. However, liquid coatings that are useful in gas chromatography (GC) are also useful at trapping analytes from gaseous samples. It is not uncommon to perform direct collection of gases from the atmosphere at the head of a GC column kept at relatively low temperature (typically $-50^{\circ}C$ to $50^{\circ}C$). The chemist must be careful, however, not to expose the column to temperatures outside its range of operation. Once the sample has been extracted/collected on the column, injection occurs by simply beginning the oven temperature programme to perform separation and analysis. Purge-and-trap techniques are another way of accomplishing this without introducing air into the GC system.

Thermal Desorption

Thermal desorption is an extraction technique which utilizes a flowing gas to extract a small heated solid or liquid sample. This process occurs during injection in GC systems and, in some cases, the approach is useful to separate thermally the analyte from the matrix for direct analysis in a flowing gas stream. The approach is not used widely in direct sample analysis, even for stable volatile and semivolatile analytes for a variety of practical reasons. For example, analyte–matrix interactions may be too strong in some cases, or matrix interferences may be too great.

Certain analytes are more prone to degradation during thermal desorption and, in multiresidue methods, these analytes may be deemed too important to sacrifice. Another potential pitfall may be that the sample size is too small to achieve the desired LOD. Thus, a liquid concentration step may be needed to increase the injected relative sample size, but then clean-up is usually required. Otherwise, thermal desorption can lead to a very rugged approach for sample introduction in GC analysis because the selectivity of the extraction matches the selectivity of the analysis. Unlike liquid sample introduction, nonvolatile components in the extract are not introduced into the GC column and the life of the chromatographic system is extended.

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Solid-Phase Microextraction (SPME)

In the late 1980s, Pawliszyn and his group at the University of Waterloo in Canada invented a technique dubbed SPME, which conveniently takes advantage of the absorption and desorption processes between gases, liquids and solids. An SPME device is a small fibre rod that has been coated with a solid or liquid phase and which is contained in a pen-like sleeve. The coated fibre is exposed to the gaseous or liquid sample and then retracted into the sleeve for brief storage. Analysis may involve thermal desorption of the fibre in a GC injection port or solvent elution of the analytes from the fibre coating into a liquid chromatographic (LC) system.

SPME is applicable to extraction of liquid samples, but it has been most noteworthy for its effectiveness in the extraction of gases. In fact, one of its modes of operation for sampling liquids is to place the fibre in the headspace above the liquid sample in an enclosed volume. The analyte will eventually partition into the headspace and then into the coating on the fibre which can be desorbed into a chromatographic system for analysis.

The major advantage of SPME is ease of use. Other advantages include low cost and avoidance of hazardous solvents. At this time, only a few fibre coatings

are available, and this has limited the selectivity of SPME, but more coatings are expected to become available. The most common coating is polydimethylsiloxane (PDMS), which is also a common phase in GC columns.

Extractions Involving Only Liquids

Liquid–Liquid Extraction (LLE)

Water extraction is a common application for multiresidue methods. Water generally contains fewer matrix interferences than solid matrices and large volumes may often be extracted to decrease LOD. Before the widespread introduction of convenient solid-phase extraction (SPE) cartridges, LLE was the method of choice for extraction of water pollutants. Dichloromethane (DCM) is the most common solvent for extraction in LLE of water because: (i) it is only slightly miscible with water, (ii) it extracts an acceptably wide range of nonpolar analytes; (iii) it possesses a low boiling point to speed evaporation/concentration steps; and (iv) it is heavier than water and thus exists as the lower phase during partitioning in a separatory funnel or LLE glassware. Of course, other immiscible solvents are also used in LLE.

Traditionally, continuous LLE is conducted using specialized glassware which passes redistilled DCM through the water for an extended period of time (16–24 h). The extract collects with the DCM in a boiling flask where the DCM is removed by distillation. Otherwise, either manual or mechanical shaking is used to speed the extraction process (at the cost of more solvent volume and effort). In most cases, sample volume is limited to 1 L by the practical nature of the extraction process and size of the glassware. SPE has virtually displaced LLE in water methods due to its greater versatility, convenience, solvent reduction and sample volume capacity.

Extractions Involving Liquids and Solids

The most common application in chemical residue analysis concerns the extraction of a solid sample using a liquid. A variety of liquid solvents are readily available to provide a medium for easy homogenization in a blending device. **Table 1** lists key properties of common liquids used in multiresidue methods. These parameters indicate the relative polarity of the solvent, volatility and miscibility with other liquids. In extraction processes, the tenet that like dissolves like (and conversely, opposites do *not* attract) is the primary consideration in choosing the extraction solvent. For example, hexane often provides a selective extraction for nonpolar analytes, and toluene may provide more selectivity for aromatic analytes. Practical considerations involving ease of evaporation, cost, safety and hazardous waste disposal also play a role in the selection of the extraction solvent. In situations involving acidic/basic analytes, pH is often the most critical property in the extraction and buffered aqueous solvents are often necessary. Another important consideration is the stability of the analytes in the extraction medium.

Soxhlet Extraction

In this technique, the sample is mixed with a dispersant and/or drying agent and placed in a permeable paper thimble. The extraction thimble is placed in a glass apparatus which is exposed to the extraction solvent. Fresh solvent enters the extraction section

Table 1 Selected properties of common solvents used in extractions

Solvent	*Polarity index*	*Dielectric constant*[a]	*Boiling point (°C)*	*Viscosity (mN s $^{-1}$ m $^{-2}$)*	*Density*[b] *(g mL $^{-1}$)*	*Solubility in water (%w/w)*
Acetone	5.1	20.7	56.2	0.337	0.791	100
Acetonitrile	5.8	37.5	81.6	0.375	0.786	100
Cyclohexane	0.2	2.02	80.7	0.980	0.779	0.01
Dichloromethane	3.1	9.08	40.7	0.449	1.326	1.6
Diethyl ether	2.8	4.34	34.6	0.245	0.713	6.89
Ethanol	5.2	24.55	78.4	1.08	0.789	100
Ethyl acetate	4.4	6.02	77.2	0.455	0.901	8.7
Hexane	0.0	1.89	69.0	0.313	0.659	0.001
Iso-octane	—	1.94	99.2	0.504	0.692	—
Methanol	5.1	32.70	64.6	0.544	0.791	100
Toulene	2.4	2.57	110.8	0.587	0.866	0.051
Water	9.0	78.30	100.0	0.890	0.998	—

[a]At 25°C.
[b]At 20°C.

from the distillation section of the apparatus. When the solvent reaches a certain level, the extract siphons into a boiling flask where the extracted components are concentrated. The solvent is boiled and redistilled to fall back into the region where the sample is contained. In this way, the Soxhlet glassware is designed to repetitively conduct a number of extractions of the matrix with fresh (redistilled) solvent each time. This process is rather time-consuming (up to 16–24 h) to achieve adequate extraction efficiencies and takes up a great deal of glassware and laboratory space. Automated Soxhlet instruments have been introduced, but the Soxhlet approach is frequently regarded in modern laboratories as archaic.

Blending and Sonication

Blending the sample with the solvent is also an old-fashioned approach, but it is very fast, convenient and inexpensive. Thus, the use of blenders, choppers, shakers, probes and other mixing devices is not likely to disappear even as newer instrumental techniques are being introduced. In the case of matrices such as clay soils that tightly retain certain analytes, sonication using a high energy probe is an alternative method that can break matrix–analyte interactions. However, due to the higher energy input involved, sonication has a greater potential for degrading analytes than simple blending, but the approach can be useful for stable analytes.

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Microwave-assisted Extraction (MAE)

MAE is a technique used in the 1990s for the extraction of organic residues in solid samples (microwave digestion has been used in the analysis of metals for several years). The approach simply involves placing the sample with the solvent in specialized containers and heating the solvent using microwave energy. The extraction process is more rapid than Soxhlet, and reduces solvent consumption, but it is more complicated and time-consuming than blending. As in the case of sonication, MAE may overcome retention of the analyte by the matrix, but analyte degradation can be a problem at higher temperatures in certain applications.

The selection of solvent, microwave energy applied and extraction time are the main parameters controlled in MAE. The user should use proper extraction vessels and equipment in MAE, because very high pressures can be generated and explosions may result if appropriate precautions are not taken. MAE instruments are available that conduct batch extractions to increase sample throughput, which is an advantage over automated instruments in other techniques that perform sequential extractions.

Pressurized Liquid Extraction (PLE)

PLE is another time-saving and solvent-reducing approach that was developed in the mid-1990s. The instrumental approach generally involves first dispersing the sample with an inert material (e.g. drying agent or sand) and placing the mixed sample in an extraction vessel. The general approach consists of introducing the solvent into the vessel followed by heating the vessel and a static extraction step (no flow). After this 0.5–20 min step, flow is initiated (dynamic extraction step) and the extract is collected in a vial. The process may be repeated if necessary to increase analyte recoveries. Although increased temperature is not a necessity in PLE, higher temperature is usually used to speed the extraction and break analyte–matrix interactions.

The order of importance of parameters for an application in PLE (and extraction in general) is typically: (1) solvent; (2) temperature; (3) time; (4) repetitions; (5) pressure. The same types of solvents can be used in PLE as in traditional approaches, but relatively viscous solvents, such as ethanol and water, can be difficult to permeate through the sample even at high pressures. Also, highly acidic and basic conditions can be damaging to instrument components, which limits the use of PLE in certain applications. The properties of solvents can change dramatically at different temperatures and pressures (the boiling point at room temperature is commonly exceeded in PLE and MAE), thus it may be possible to replace potentially more hazardous solvents with more benign solvents. Unfortunately, physicochemical properties of many common solvents are not yet known at the elevated temperatures and pressures possible in PLE and MAE.

Solid-Phase Extraction

SPE, sometimes referred to as liquid–solid extraction, is a popular technique for the isolation and separation of analytes from a liquid matrix. SPE columns, packed with small quantities of various chromatographic sorbents, are commercially available. SPE columns may contain polar sorbents such as silica, Florisil or alumina for normal-phase separations, and nonpolar bonded silica phases or polymers for reversed-phase separations. One of the most widely used types of SPE columns is packed with nonpolar octadecylsilyl-derivatized silica, or C_{18}. When liquids such as water, plasma and in some cases, milk, are eluted through C_{18} SPE columns, nonpolar organic compounds such as certain pesticides, drugs or industrial pollutants will be adsorbed onto the column. These adsorbed analytes can be later eluted from the column with a relatively small amount of solvent.

SPE discs containing C_{18} are widely used for the isolation of contaminants from water. Large volumes (litres) of water can be fairly rapidly eluted through the discs, and organic compounds, such as OC pesticides, PCBs and PAHs will be retained. These trapped organic compounds can then be eluted from the SPE discs with organic solvents. In many cases, the choice of solvent, pH and SPE phase provides clean-up of matrix components during the extraction process.

Matrix Solid-phase Dispersion (MSPD)

MSPD is an extraction technique that entails mixing a sample of tissue or milk with an SPE sorbent. Stephen Barker and his group at Louisiana State University first developed the concept in the late 1980s. Typically, a small quantity (0.5 g) of liver, muscle, fat or milk is homogeneously dispersed with 2 g of C_{18} silica in a mortar and pestle. The C_{18} silica will disrupt the cells and disperse the contents over a large surface area, thereby exposing the entire sample to the extraction process. This homogeneous dispersion is then placed in a column, and the various components of the dispersion can be eluted from the column with a range of solvents. For example, lipids or fats can first be eluted from the column with hexane, while drugs, which are more polar, can be eluted with more polar solvents such as ethyl acetate and/or methanol. Thus, extraction and clean-up can be performed in a convenient procedure. Disadvantages of MSPD include the small sample size and potentially high cost of the solid-phase material.

Extractions Involving Supercritical Fluids

Supercritical Fluid Extraction (SFE)

SFE is an instrumental approach not unlike PLE, except a supercritical fluid is used as the extraction solvent rather than a liquid. SFE and PLE employ the same procedures for preparing samples and loading extraction vessels, and the same concepts of static and dynamic extractions are also pertinent. SFE typically requires higher pressure than PLE to maintain supercritical conditions and, for this reason, SFE usually requires a restrictor to better control flow and pressure of the extraction fluid. CO_2 is by far the most common solvent used in SFE due to its relatively low critical point (73 atm and 31°C), extraction properties, availability, gaseous natural state and safety.

A major advantage of SFE over liquid-based methods is that the extraction solvent becomes a gas after extraction and the analytes are conveniently concentrated in the collecting medium (solid-phase trap or liquid). Liquid extraction methods nearly always require a concentration step after extraction. Another key advantage of SFE is that the density of the supercritical fluid and other physicochemical properties can be dramatically altered through control of temperature and pressure. This permits a somewhat higher degree of selectivity and versatility in the extraction process without having to use different solvents. In some cases, SFE can eliminate post-extraction clean-up steps, or at least make clean-up using SPE exceptionally convenient by using the SPE sorbent as a trapping medium in SFE. Due to its many practical advantages, SFE may be considered the first choice for extraction if it is able to meet the needs of the application.

However, SFE also has several disadvantages which have delayed the widespread implementation of the approach. The higher selectivity of SFE limits the range of analytes that can be extracted under the same conditions. Furthermore, SFE can have difficulty in overcoming analyte–matrix interactions in certain applications (soils in particular). Organic solvents (and water), often called modifiers in SFE, are sometimes added to the supercritical fluid to increase the polarity range of the extraction process and to help overcome analyte retention in the matrix. Other problems with SFE include the high cost of automated instruments, relatively small sample sizes and more involved method development process. SFE has been demonstrated to be effective in the extraction of a variety of residues from a variety of matrices, but it remains to be seen if the technique can overcome its drawbacks and become more widely implemented.

Conclusions

The analytical range of the overall analysis cannot exceed the analytical range of the extraction process, and in the case of multiresidue methods it is not uncommon to have a wide polarity range of analytes. For this reason, the use of rather exhaustive extraction conditions has been the traditional approach in multiresidue methods. The cost of a wide scope of analytes is often reduced selectivity, solvent-consuming and longer extractions, and additional clean-up of extracts. Ideally, however, the selectivity of the extraction process should match the polarity range of the targeted analytes, and no further clean-up would be required prior to analysis. Modern techniques may permit the realization of the ideal extraction process which is fast, automated, precise, efficient and safe. Furthermore, the variety of solvents and solid-phase sorbents, in combination with temperature and pressure control of modern instruments, can give the

chemist the ability to achieve the desired selectivity in a single, convenient extraction procedure.

Due to the additional parameters of temperature and pressure that modern instrumental techniques provide, it has become more difficult to compartmentalize extraction techniques based on whether the extraction fluid is a dense gas, liquid, supercritical fluid, or combination thereof. Strictly speaking, pressurized fluid extraction PFE includes all types of pressurized extractions independent of the solvent's state of matter. Subcategories of PFE include SFE and PLE, but instrument companies have confused the terminology by marketing PLE as accelerated solvent extraction (ASE^{TM}) and enhanced solvent extraction (ESE). Other scientists have developed other terms to describe extraction techniques, such as enhanced fluidity extraction, which connotes a mixture of gas, liquid and/or supercritical fluid, and subcritical water extraction, which is meant to represent PLE using water at high temperatures. However, the unifying principles of extraction are the same no matter what instrument-makers or scientists may call a particular approach.

See also: **II/Chromatography: Gas:** Headspace Gas Chromatography. **Extraction:** Solid-Phase Extraction; Solid-Phase Microextraction; Solvent Based Separation; Supercritical Fluid Extraction. **III/Environmental Applications:** Soxhlet Extraction; **Microwave-Assisted Extraction: Environmental Applications. Solid-Phase Matrix Dispersion: Extraction.**

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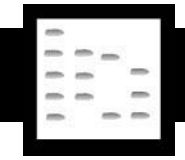

High-Speed Countercurrent Chromatography

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In high speed countercurrent chromatography (HSCCC) a sample is partitioned between two nonmiscible liquid phases. One phase is held stationary by a centrifugal force (applied by spinning the separation element at high speed), while the second phase is pumped through the apparatus, hence the alternative term for the process centrifugal partition chromatography (CPC).

Unlike high performance liquid chromatography (HPLC), in which the stationary phase occupies 5–7% and the mobile phase about 75% of the column, the relative proportions in HSCCC are 50–75% for the stationary phase and 20–50% for the mobile phase. As a consequence, large sample loads are possible with HSCCC. Another important advantage of the absence of a solid support is that irreversible adsorption is avoided. There is total recovery of the injected sample and tailing is minimized. HSCCC is thus of special importance for the separation of sensitive and easily degraded samples. Although the efficiency of HSCCC separations is lower than that encountered in HPLC, the optimization of selectivity is the great advantage offered by the former technique.

The potential of HSCCC is further shown by the possibility of applying gradients for separations.